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QUALITATIVE AND QUANTITATIVE STUDY OF PHYTOCHEMICALS IN *MUNTINGIA CALABURA* L. LEAF AND FRUIT

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ABSTRACT

Muntingia calabura L is a fast growing medicinal plant, attracts fruit eating birds such as flower peckers. The timbers are lightweight, durable and used in carpentry. Fruit from Muntingia is also harvested for export overseas. The leaves and fruits were studied for its phytochemical analysis both qualitatively and quantitatively and also for its behavior with aqueous extract powders, fluorescence, nutrients. Among the phytochemicals assessed, the carbohydrate, glycosides, tannin, phenolic compounds, proteins and aminoacids showed strong reactions, whereas, moderate reaction was observed with rest of the phytochemicals. Positive result was observed with flurosence study. The total yield obtained from 15gm powder was 09.95% with leaf and 16.01% with fruit. The carbohydrate content was found to be higher with leaf (204.0±3.46mg/g carbohydrate) when compared to fruit (75.33±4.61mg/g carbohydrate). But, the protein content was higher with fruit extract (6.44±0.15mg/g protein) on comparison with leaf extract (2.04±0.15mg/g protein). Moderate amount of aminoacids content was observed with leaf and fruits. The behavior of the extract powder was positive for alkaloids, proteins, flavonoids, anthroquinone.

KEY WORDS: Aminoacids, Aqueous extract, Leaf, Muntingia calabura, Protein.

INTRODUCTION

Muntingia calabura L., (Family: Muntingiaceae) is a fast-growing slender tree, native to the American continent and is commonly cultivated in warm areas of Asian region. Other names of this plant are straw berry tree, Jamaican cherry (English), Chinese cherry (or) Japanese

cherry (India) and cherry chettu (Telugu) [1]. The leaves are rich in flavanoidal compounds like flavones, flavanones, flavans, and biflavans as the major constituents, possessing antidiabetic and cytotoxic activities [2],[3]. Peruvian claim that this plant possess several medicinal values [4], [5]. The leaves are used to treat headache, cold, gastric ulcer, or to attenuate the prostate gland swelling [4],[5],[6]. Other parts like roots, flowers possess medicinal values in Vietnam and Philippines, and used as emmenagogue, abortifacient, antidyspeptic, antispasmodic, and diaphoretic, and to treat headaches, dyspepsia, and spasm [7]. Reports are there for its anti-tumor, antinociceptive, anti-inflammatory, anti-pyretic, antibacterial, antiproliferative and antioxidant, antihypertensive, antiulcer and antistaphyloccocal activities [7-16]. In Mexico, the fruits are eaten and sold in markets. Their fruits are processed in to jams, leaves are used for making tea. In Brazil, the trees are planted along river banks. Since, this plant is a medicinal plant, the present work has been designed for the evaluation of phytochemicals qualitatively, quantitatively in leaf and fruit.

MATERIALS AND METHODS

Sample collection

The samples Muntingia calabura leaf and fruits were collected from the campus of Periyar University, Salem, Tamil Nadu, India, during the month of March - April, 2014. The collected leaves and fruits were cleaned thoroughly and dried under the shade. Once the drying process is complete, the dried leaves and fruits were ground to powder using blender for further use.

Aqueous extract preparation

Aqueous extract was prepared by dissolving 15g of powdered Muntingia calabura leaf and fruits in 200ml of distilled water. The mixture was heated on a hot plate with continuous stirring at 30-40°C for 20minutes. Then the water extract was filtered through filter paper. The filtrate was kept in a beaker and allowed to dry by heating in a boiling water bath. The gummy residue obtained was used for the analysis of percentage yield, and the remaining marc left was extracted with water and used for Qualitative analysis.

Phytochemical analysis

The extract was tested for the presence of bioactive compounds by adopting standard procedures [17], [18] fluoresence analysis [19], behaviour of aqueous extract powder with different chemical reagents [20].

Test for carbohydrate

Molisch's test: To the extract added few drops of alcoholic alpha napthol solution, few drops of concentrated Sulphuric acid along the sides of test tube. Positive result gives purple or violet colored ring at the junction. Fehlings test: To the extract added equal amount of Fehlings A and B solution, heat the tubes in a boiling water bath. Brick red precipitation of cuprous oxide is formed, if reducing sugar is present. Benedicts test: To the extract add Benedicts reagent, the tubes were heated in a boiling water bath. Red precipitation indicates positive result.

Test for alkaloids

Wagners test: To the extract add few drops of iodine solution in potassium iodide. Reddish brown precipitate shows positive result. Hagers test: To the extract add few drops of saturated solution of picric acid. Yellow colour precipitation signifies positive result.

Test for steroids and sterols

Libermann-Burchard test: To the extract add 2ml chloroform, 10 drops of acetic anhydride, 2 drops of concentrated sulphuric acid. Bluish red to cherry red colour in chloroform layer shows positive result. Salwoski test: To the extract add few drops of chloroform, concentrated sulphuric acid. Bluish red to cherry red colour.

Test for Glycosides

Legal test: To the extract added pyridine, sodium nitroprusside. Positive result shows pink red colour. Baljet test: To the extract add picric acid. Appearance of orange color signifies positive result.

Test for saponins

Foaming test: Foams produces when the extract is shaked with water.

Test for flavonoids

Shinoda test: To the extract added magnesium turnings, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicates positive result. Zinc hydrochloride test: To the extract added zinc dust, 1-2 drops of concentrated hydrochloric acid. Appearance of red color indicates positive result.

Test for tannin and phenolic compounds

Ferric chloride test: To the extract add ferric chloride. Formation of greenish black colour shows positive result. Potassium dichromate test: To the extract add potassium dichromate solution. Positive result is confirmed by a formation of brown precipitate. Gelatin test: To the extract add 1% gelatin solution containing 10% sodium chloride gives white precipitation.

Test for protein and amino acids

Biuret test: To the extract added 4% sodium hydroxide, few drops of 15% copper sulphate gives purple colour. Ninhydrin test: Bluish violet colour forms when a solution of ninhydrin and extract mixture was heated. Heat test: Protein coagulation shows positive result when test solution is heated on a boiling water bath.

Test for fixed oil

Copper sulphate test: Blue colour forms when the extract is mixed with 1ml of 1% copper sulphate and 10% sodium hydroxide.

Quantitative analysis of phytonutrients

Total carbohydrates [21], proteins [22], aminoacids [23] were performed according to the standard prescribed methods.

Estimation of carbohydrate

The total carbohydrate was estimated by anthrone method**.** 1mg of powdered Muntingia calabura leaf and fruits was hydrolysed to simple sugars by keeping it in a boiling water bath for three hours with 5ml of 2.5N Hcl and cool to room temperature. After neutralizing, the contents were centrifuged and 0.1 ml of supernatant was used for the analysis. To the sample add 4ml of anthrone reagent and contents were heated in a boiling water bath for 8 minutes. The tubes were cooled and read at 630nm using spectrophotometer Schimadzu Model - UV 1800. The standards were developed with glucose. Standard graph plotted was used to find out concentration of glucose present in unknown/ sample.

Estimation of protein

The total protein was estimated by Lowry's method. To 0.1ml of extract added 2ml of alkaline copper reagent, mixed well and incubated for 10minutes. After the incubation period 0.2ml of folin ciocalteau reagent (diluted in the ratio of 1: 2) was added and allowed for 30minutes incubation, then read at 660nm using spectrophotometer Schimadzu - Model UV

1800. The standards were developed with Bovine serum albumin. Standard graph plotted was used to find out concentration of protein present in unknown/ sample.

Estimation of aminoacids

The amino acid was estimated by Ninhydrin method. To 0.1 ml of sample added 1 ml of ninhydrin solution dissolved in ethanol. Cover the test tube with a piece of paraffin film to avoid the loss of solvent due to evaporation. With gentle stirring, react at 80-100ºC for 4-7 minutes. Cool the test tubes and the colour developed was read at 570nm. Tyrosine was used for developing standards.

Statistical Tool

Each experiments were carried out in triplicate and the results are given as the mean ± standard deviation. The Mean and Standard deviation (S) was calculated by using the following formula: Mean = Sum of x values / n (Number of values), $S = \frac{\sqrt{\Sigma(x-M)^2}}{n-1}$

RESULTS AND DISCUSSION

The percentage recovery of the aqueous extract obtained was expressed in Table 1.

Table.1 Percentage yield of Muntingia calabura L leaf, fruit aqueous extract

S.No	Name of the samples used	Weight taken for extraction	Initial weight of the beaker	Final weight of the beaker	Weight of the extract	% recovery
1.	Muntingia calabura leaf powder	15g in 200ml	176.1638	177.6563	1.4925	09.95
2.	Muntingia calabura fruit powder	15g in 200ml	172.0553	174.4582	2.4029	16.01

The yield percentage was found to be 9.95 for leaf sample and it was found to be 16.01 for fruit sample when 15gm of each sample was used for extraction.

Analysis of aqueous extract leaf, fruit powder for its behavior

Table.2 Behaviour of Muntingia calabura leaf, fruit powder with different chemical reagents

S.No	Tests	Observation Muntingia calaburaleaf	fruit	Inference Muntingia calabura leaf	fruit
1.	Powder+Picric acid	Yellow color	Yellow color	Presence of alkaloid	Presence of alkaloid
2.	Powder+Conc. H_2SO_4	Reddish brown color	Reddish brown color	Presence of steroids	Presence of steroids
3.	Powder+ Aqueous $FeCl_3$	Green color	Green color	Presence of flavonoids	Presence of flavonoids
4.	Powder+Iodine solution	Brown color	Brown color	Absence of starch	Absence of starch
5.	Powder+ Ammonia solution	Brown colour	No blood red colour	Presence of anthroquinone	Absence of anthroquinone
6.	Powder+ Aq. 5% KOH	Brown color	Brown color	Presence of anthroquinone	Presenceof anthroquinone
7.	Powder + NaOH	Yellow color	Yellow color	Presence of flavonoids	Presence of flavonoids
8.	Powder+ Aqueous $AgNO_3$	White precipitate	White precipitate	Presence of protein	Presence of protein

The results of aqueous extract powder studied for its behaviour with different chemical reagents are tabulated in Table.2. The results obtained showed, that the leaf and fruit extract powders were found to be positive for alkaloids, steroids, flavonoids, anthroquinone, protein.

Fluoresence analysis

Table.3 Fluoresence analysis of aqueous leaf and fruit extract of Muntingia calabura

S.No	Name of the aqueous extract	Day light	UV light
1.	Muntingia calabura leaf	Pale cloudy brown	Orange fluoresence
2.	Muntingia calabura fruit	Pale bottle green	Orange fluoresence

The results of flurosence analysis showed orange fluoresence with leaf as well as fruit of Muntingia calabura. (Table.3)

Phytochemical analysis: The results of phytochemical analysis are shown in Table.4.

Table.4 Phytochemicals present in Aqueous extract of Muntingia calabura leaf and fruit

S.No	Name of the test	Results	
		leaf	fruit
1.	**Test for carbohydrate**		
	a)Molisch's test	+++	+++
	b)Fehlings test	+++	+++
	c)Benedicts test	+++	+++

2.	**Test for alkaloids** a)Wagners test b)Hagers test	 ++ ++	 ++ ++
3.	**Test for steroids and sterols** a)Libermann - Burchard test b)Salwoski test	 +++ ++	 +++ -
4.	**Test for Glycosides** a)Legal test b)Baljet test	 +++ +++	 +++ +++
5.	**Test for saponins** Saponin test	 ++	 -
6.	**Test for flavonoids** a)Shinoda test b)Zinc hydrochloride test	 + ++	 - -
7.	**Test for tannin and phenolic compounds** a)Ferric chloride test b)Potassium dichromate test c)Gelatin test	 +++ ++ +	 +++ +++ +
8.	**Test for protein and amino acids** a)Biuret test b)Ninhydrin test	 +++ -	 +++ +++
9.	**Test for fixed oil** a)Copper sulphate test	 +	 +

+Slight changes, ++ Moderate, +++ Stronger reactions

The results of phytochemical analysis of Muntingia calabura leaf and fruit is shown in Table. 4. The results were found to be positive for leaf but the fruit showed positive results for most of the tests performed except saponin and flavonoid. The result obtained might be because of the stage of the fruit at which it was collected.

Table.5 Nutrient content of leaf, fruit of Muntingia calabura aqueous extract

S.No	Nutrients	Calculated nutrient content
1.	**Muntingia calabura leaf** Total carbohydrate Total protein Amino acids	 204.0±3.46mg/g carbohydrate 002.04±0.15mg/g protein 001.41 ±0.07mg/g amino acids
2.	**Muntingia calabura fruit** Total carbohydrate Total protein Amino acids	 75.33±4.61mg/g carbohydrate 06.44±0.15mg/g protein 00.88±0.07mg/g amino acids

Values are Mean ± SD for three experiments

Phytonutrient Analysis

The phytonutrients estimated were tabulated in Table.5.

The carbohydrate content was found to be higher with leaf (204±3.46mg/g) when compared to fruit (75.33±4.61mg/g), but the protein content was lower for leaf (2.04±0.15mg/g) and showed higher protein content for fruit (6.44±0.15mg/g), Similarly, the amino acid content was found to be moderate with both the samples studied. The air pollution tolerance index and antioxidant activities in fresh aqueous leaf extract was reported by Krishnaveni et.al. [24], [25], [26].

CONCLUSION

Muntingia calabura, a medicinal plant having huge therapeutic application has to be assessed for its properties in order to find a panacea for diseases. From the results of our study, we can conclude that leaf and fruits are the richest source of phytochemicals which could be used to serve the community well towards the betterment. Further research is needed in this regard in purifying active principle from the leaf and fruit.

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